Vaccine 32 (2014) 4660–4664

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Reversion to virulence of a subtype B avian metapneumovirus vaccine: Is it time for regulators to require availability of vaccine progenitors?

M. Cecchinato[a], E. Catelli[b,*], C. Lupini[b], E. Ricchizzi[b], S. Prosperi[b], C.J. Naylor[c]

[a] Department of Animal Medicine, Production and Health, University of Padua, Viale dell'Università, 16, 35020 Legnaro (PD), Italy
[b] Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra, 50, 40064 Ozzano Emilia (BO), Italy
[c] Department of Infection Biology, University of Liverpool, Leahurst Campus, Neston, Cheshire CH64 7TE, United Kingdom

ARTICLE INFO

Article history:
Received 20 March 2014
Received in revised form 20 May 2014
Accepted 6 June 2014
Available online 30 June 2014

Keywords:
Avian metapneumovirus
Vaccine progenitor availability
Environmental vaccine identification
Reversion to virulence
Farm disease

ABSTRACT

Empirically derived live avian metapneumovirus (AMPV) vaccines developed during the late 80s and early 90s have generally performed well in controlling turkey rhinotracheitis. Nonetheless, unstable attenuation was previously demonstrated in an AMPV subtype A vaccine. Until now this had not been investigated in subtype B vaccines due to lack of any similar availability of a vaccine progenitor or its sequence. The publication of the full genome sequence for the VCO3 vaccine progenitor facilitated a conclusive investigation of two AMPVs isolated from poults on a farm which had been vaccinated with VCO3 derived vaccine. Full genome sequencing of the isolates and their comparison to sequences of the vaccine and its progenitor, confirmed their vaccine origin. After determining the absence of extraneous infectious agents, one of these virus isolates was inoculated into 1-day-old turkeys in disease secure isolators and shown to cause disease with a severity similar to that caused by virulent field virus. This suggests that instability in live AMPV vaccines may be generalized and highlights the need for availability of vaccine progenitor sequences for the field assessment of all live viral vaccines.

1. Introduction

Avian metapneumovirus (AMPV) (genus Metapneumovirus, family *Paramyxoviridae*) is found as four subtypes (A, B, C and D) [1–3]. The viruses cause upper respiratory tract infection in turkeys (Turkey rhinotracheatis (TRT)) and other avian species, including chickens [4–7] and pheasants [8–10]. Large economic losses occur in unprotected birds, especially when secondary pathogens become involved [11–13]. Subtypes A and B dominate in Western Europe [14].

Live empirically derived attenuated vaccines were developed during the late 80s and early 90s in France and UK which generally controlled disease. However from the outset occasional poor or sub-optimal performances were observed; especially when applied in multiage sites, where vaccine of a different subtype had been used [15,16] or when vaccine application was suspected to be suboptimal. For subtype A virus infection, some disease was found to be caused by reversion to virulence of subtype A vaccine [17,18]. More recently evolution of virus in response to a dominant vaccine has been reported as a further factor [19,20].

After one day old subtype A vaccination on turkeys farms, it had been observed previously that virus of the vaccine subtype could be detected at two to five weeks of age [15]. Similar virus was later shown to cause disease [17] and a single amino acid change was shown to cause the virulence increase [21]. While unstable attenuation was proved for a subtype A vaccine, similar studies proving reversion of subtype B vaccines were not reported due to inaccessibility of vaccine progenitors or their sequences. This situation changed after the recent publication of the full VC03 vaccine progenitor sequence [22]. Prior to this, an Italian study detected subtype B viruses closely genetically related to the B subtype vaccine applied two to four weeks previously [19] and later Italian farm studies found respiratory signs and/or increase of mortalities where AMPV subtype B vaccine derived virus was detected [23,24]. Furthermore, another study [25] proposed that subtype B vaccine reversion would best explain their disease findings.

The current study made use of full genome sequencing and the available sequencing data to identify subtype B vaccine derived virus from the field, then determined whether these viruses had

Abbreviations: AMPV, avian metapneumovirus; PCR, polymerase chain reaction; TRT, turkey rhinotracheatis; dpi, days post infection; IBV, infectious bronchitis virus; TOC, tracheal organ cultures.

* Corresponding author. Tel.: +39 051 2097080; fax: +39 051 2097039.
E-mail address: elena.catelli@unibo.it (E. Catelli).

http://dx.doi.org/10.1016/j.vaccine.2014.06.030
0264-410X/

reacquired virulence. Two Italian subtype B AMPV strains, isolated four weeks after B vaccine application, were sequenced in their entirety and compared to vaccine and vaccine progenitor sequence, in order to identify the presence of vaccine specific mutations together with potential virulence associated nucleotide changes. Evidence of reversion to virulence of one of these vaccine derived subtype B isolates was investigated by experimental infection of one-day-old turkeys.

2. Materials and methods

2.1. Full genome sequencing and comparison to AMPV subtype B strains

2.1.1. Virus strains and sequencing of full genome

During longitudinal studies performed in two Italian farms, two AMPV strains, IT/Ty/129-08/04 and IT/Ty/132-08/04, were isolated from four-week-old turkeys showing respiratory disease [19]. Viruses were isolated on embryo tracheal organ cultures (TOC) [26] and confirmed as AMPV subtype B by real time PCR [27]. In the hatchery, live subtype B AMPV vaccine (strain VCO3/50) had been applied at one day of age by course spray to all poults.

To confirm vaccine origin, the genome of the two strains together with a commercial live AMPV B subtype vaccine (strain VCO3/50) derived from VCO3/60616, were sequenced. Virus genomes were copied to DNA and amplified in their entirely [24]. Amplified DNA products were purified using the Wizard1 SV Gel and PCR Clean-Up System (Promega). Sequencing was performed at Bio-Fab Research (Rome, Italy) with the 3730xl DNA analyzer (Applied Biosystems, USA). Nucleotide sequences were edited and assembled using BioEdit software version 7.0.9, then aligned with and compared to the vaccine progenitor (GenBank Accession number AB548428.1) using CLUSTAL W web interface [28]. If any region of the sequence showed a difference between the strains analyzed, the amplification and sequencing were repeated using a new RNA extraction.

2.2. Virulence assessment of a vaccine-derived

After eliminating the possibility of contamination with other infectious agents (Newcastle disease virus, influenza, mycoplasmas and bacteria), virulence of IT/Ty/129-08/04 was determined by assessment of clinical signs, following inoculation of 1-day-old poults housed in a disease secure isolator. Two identical control groups in two further isolators were given either the vaccine (strain VCO3/50) or uninfected medium.

2.2.1. Viruses and doses inoculated

Isolate IT/Ty/129-08/04 was titrated in tracheal organ culture and end point was calculated [29]. For inoculation, IT/Ty/129-08/04 and vaccine were used at doses of 3.3 Log_{10} ID50 per poult. The vaccine dose represented a 10 times overdose compared to the manufacturer's recommendation.

2.2.2. Poults

Unvaccinated 1-day-old poults were obtained from a commercial turkey hatchery which enforced a high level of biosecurity.

2.2.3. Experimental design

Forty two 1-day-old commercial turkeys were housed in three positive pressure isolators with 14 poults in each. Poults in isolators one, two and three were inoculated with AMPV IT/Ty/129-08/04, vaccine and TOC medium respectively. The total volume of inoculum for each bird was 200 μl, divided in two equal parts, so that 100 μl was applied into the nostril and 100 μl dropped onto the eye. Clinical signs were observed daily up to 14 days post infection (dpi).

2.2.4. Monitoring clinical signs

Clinical signs were scored as previously described [30] and outlined below.

0, no signs;
1, clear nasal exudate;
2, turbid nasal exudate;
3, swollen infra-orbital sinuses and/or frothy eyes.

Moderate nasal pressure was applied to facilitate extrusion of exudates.

2.2.5. Statistical analysis

The distribution of clinical scores within groups were tested using the Kruskal–Wallis non parametric one-way ANOVA. The differences between groups were tested using the Mann–Whitney test. A p value <0.05 was considered statistically significant.

3. Results

3.1. Full genome sequencing and comparison of AMPV subtype B strains

Vaccine and progenitor differed only at 17 positions (Table 1). The complete sequencing of the two Italian strains showed that both were identical to the vaccine at the 17 positions where the vaccine and its progenitor differed (Table 1). Both IT/Ty/129-08/04 and IT/Ty/132-08/04 differed from vaccine at 7 further positions (Table 2) while IT/Ty/132-08/04 had an additional single mutation. Mutations at the 7 positions arose during the reversion to virulence and some of these must have been responsible for the reversion to virulence.

3.2. Virulence assessment of a vaccine-derived virus

The daily mean clinical scores for each group are shown graphically in Fig. 1. Virus IT/Ty/129-08/04, resulted in clinical signs in all the inoculated birds from day 5. Swollen sinuses and frothy eyes were observed starting from day 6. Birds in the other two groups showed only very minor signs. The mean cumulative scores were 11.5, 1.07 and 0.64 for IT/Ty/129-08/04, vaccine and control groups respectively.

A statistically significant difference in clinical scores was found between the IT/Ty/129-08/04 inoculated group and the vaccine group ($U = 9993$; $p < 0.001$). There was also a significant difference between the IT/Ty/129-08/04 inoculated group and control group ($U = 9597$; $p < 0.001$). The difference between the vaccine and control groups was not significant ($p = 0.370$).

4. Discussion

Live AMPV vaccines have been in mass use for over 20 years, during which time they have played an effective role in the control of disease in chickens and turkeys. While problems have been encountered with respect to vaccine subtype selection, virus evolution avoiding a dominant vaccine and reversion to virulence, these have probably justifiably been regarded as infrequent events. However in our opinion, an imbalance in perception concerning reversion to virulence of live AMPV vaccines has developed since it was first reported. In some quarters, AMPV reversion to virulence has been assumed to be associated only with one particular subtype A vaccine [17,18]. Only one vaccine had been investigated